

CLAIMS

1. A chimeric photoprotein obtained by replacing a region of Obelin protein comprised between the first and the second calcium binding sites with
5 a corresponding region of a photoprotein selected from Clytin, Aequorin, Thalassicolin, Mitocromin, Mnemiopsin and Berovin.
2. A chimeric photoprotein according to claim 1, wherein said corresponding region within the selected photoprotein matches Obelin sequence in respective sequence alignments with the exception of at least 1
10 amino acid residue.
3. A chimeric photoprotein according to claim 2, wherein said corresponding region within the selected photoprotein matches Obelin sequence in respective sequence alignments with the exception of at least 5 amino acid residue.
- 15 4. A chimeric photoprotein according to claim 3, wherein said corresponding region within the selected photoprotein matches Obelin sequence in respective sequence alignments with the exception of at least 10 amino acid residue.
5. A chimeric photoprotein according to claim 1, wherein said region is
20 from residue 42 to 122 of Obelin protein sequence.
6. A chimeric photoprotein according to claim 5, wherein said region extends from residue 50 to 94 of Obelin protein sequence.
7. A chimeric photoprotein according to claim 6, in which residues 50 to 94 of Obelin protein are replaced with a fragment of Clytin sequence
25 extending from residue 53 to 97.
8. A chimeric photoprotein according to claim 7, having the amino acid sequence of SEQ ID N. 3.
9. A chimeric photoprotein according to claim 1, further comprising one

or more amino acid substitutions at positions 55, 66, 67, 73, 74, 75, 78, 83, 84, 87, 89 and 94 of Obelin sequence.

10. A fusion protein containing the photoprotein of claims 1-9.

11. A conjugation product between a photoprotein according to claims 1-6
5 and a molecule for analytical, diagnostic or therapeutic use.

12. An isolated nucleic acid molecule encoding a chimeric photoprotein according to claims 1-9.

13. An isolated nucleic acid molecule according to claim 12, encoding the protein of claim 8, having a sequence selected from SEQ. ID N. 4 and SEQ ID
10 N. 5.

14. The use of a chimeric photoprotein according to claims 1-9, in combination with a luciferin substrate, for the detection of calcium ions.

15. The use according to claim 14, wherein said luciferin substrate is coelenterazine.

16. The use according to claims 14-15, for the quantitative determination of calcium ions.

17. The use according to claims 14-15, for the determination of intracellular calcium concentration.

18. A host cell bearing a nucleic acid molecule according to claims 12-13.

20 19. The cellular host of claim 18, which is selected from bacterial, yeast, fungal, plant, insect and animal cells.

20. A method for producing a photoprotein, which comprises growing the host cell of claims 18-19 in conditions suitable for photoprotein expression, and recovering the expressed protein.

25 21. A method for the screening of biologically active molecules, which comprises exposing a cellular host according to claims 18-19 to a definite amount of said molecules and detecting any variation of intracellular calcium concentration.

22. A method according to claim 21, wherein the host cell is transfected with a heterologous G-protein coupled receptor or ion channel.

23. The use of a conjugation product according to claim 11 in a competitive solid-phase immunoassay for determining the amount of said molecule in

5 biological samples.

24. A Bioluminescence resonance energy transfer (BRET) system, comprising a fluorescent protein and the photoprotein of claim 8.